Genomics and Transcriptomics Analysis of Metal Accumulator Plants in Brassicaceae

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Abstract

The genus Brassica contains a wide range of diploid and amphipolyploid species including some of the economically high valuable vegetables and oilseed crops used worldwide. The major industrial and food crops in Brassica are the closest relatives to the model plant Arabidopsis thaliana, and hence are major beneficiaries from the vast data of genomics and molecular genetics available in the database of Arabidopsis thaliana. Extensive genetic and molecular analyses have been undertaken for the six cultivated Brassica species. The four closely related crop species B. rapa (AA, 2n=20), B. juncea (AABB, 2n=36), B. napus (AACC, 2n=38), and B. carinata (BBCC, 2n=34) provide about 12% of the worldwide edible oil supply. The other two species B. nigra (BB, 2n=16) and B. oleracea (CC, 2n=18) provide many vegetables for healthy human diet having a valuable source of dietary fiber, vitamin C and other anticancer compounds. The comparative mapping between Arabidopsis thaliana and the well known oil crops in Brassicaceae, coupled with the basic knowledge of mutation based functional analysis in Arabidopsis thaliana and QTL mapping in Brassicas, could greatly contribute towards a better understanding of the genetic architecture for the conserved as well as the evolved traits of agronomic value of crop plants in Brassicaceae. Brassica nigra has the second smallest genome size (~ 632 Mbp) among the six cultivated species of Brassica. Approximately 25% of the documented metal hyper accumulating species are members of the Brassicaceae and some of them are being used for phytoremediation. The super metal accumulating capacity of Arabidopsis halleri and Thlaspi caerulescens have been well documented. Because of their slow growth and low biomass, other fast-growing and high biomass brassica crop plants, for example Brassica juncea and Brassica nigra have been evaluated for their ability to hyper accumulate metals from contaminated soils.

The Diyabeker ecotype of B. nigra collected from southeastern part of Turkey was found to be hyperaccumulator of Cu. We carried out the comparative transcriptome analysis in order to find out the expression level of metal induced genes and transcriptome changes both in low and high Cu treated plants. Microarray analysis showed that some of the genes were highly expressed (several hundred fold) with Cu treated plants compared to control. Our microarray data using Affymetrix GeneChip Arabidopsis Genome Array (ATH1-121501 Genechip) indicate that possibly several genes including the genes in glutathione pathway, metal ATPase and ABC transporters are involved in metal tolerances in this ecotype.

1.INTRODUCTION

The Brasscicaceae family (formerly Cruciferae) consists of approximately 375 genera and 3200 species of plants, commonly known as the mustard family. Brassica contains about 100 species, including rapeseed, cabbage, cauliflower, broccoli, Brussels sprouts, turnip, various mustards and weeds (Warwick and Black, 1991). The cultivated Brassica species are the group of crops most closely related to Arabidopsis thaliana. Chromosome numbers in the Brassicaceae vary from 2n = 8 to 2n = 256 (Lysak et al., 2005) A. thaliana, with 2n = 10, has one of the smallest chromosome numbers, an advanced character representing reduction from its ancestors in the clade including A. lyrata and Capsella rubella (both 2n = 16).

The species typically termed the "diploid" Brassica species, B. rapa (n = 10), B. nigra (n = 8) and B. oleracea (n = 9) contain the A, B and C genomes, respectively. Each pairwise combination has hybridized spontaneously to form the three allotetraploid species, B. napus (n = 19, comprising A and C genomes), B. juncea (n = 18, comprising A and B genomes) and B. carinata (n = 17, comprising B and C genomes). The genome of B. rapa is the smallest, at ca. 500 Mb (Arumuganathan and Earle, 1991), and a genome sequencing project is under way, with both sequences and sequence annotations in the public domain http:// brassica.bbsrc.ac.uk/.

2.Transcriptional regulation in response to Cu treatments to Brassica nigra

Arabidopsis thaliana has become a model molecular genetics system because of its extensive genetic characterization, compact genome, known genomic sequence and compact growth habit, and the availability of a wide variety of tools for its molecular genetic manipulation. However, it does not accumulate metal. Interestingly, approx. 25% of the documented metal hyperaccumulating species are the members of the Brassicaceae (Peer et al., 2006).

The heavy metal accumulating species Brassica nigra and B. juncea have received attention due to its possible use for phytoremediation of heavy metal-polluted soils (Muthukumar et al 2007, Memon et al 2008). Tolerance to metals is based on multiple mechanisms such as cell wall binding, active transport of ions into the vacuole and formation of complexes with organic acids or peptides (Memon and Schroder, 2009). Here, one of the most important mechanisms for metal detoxification in plants appears to be chelation of metals by low molecular weight proteins such as metallothioneins and a family of peptide ligands, the phytochelatins.

In the last decade, the tremendous developments in molecular biology and the success of genomics have highly encouraged studies in molecular genetics, mainly transcriptomics, for the identification of the functional genes implied in metal tolerance in plants (Hammond et al., 2006; Muthukumar et al., 2007). These studies have already succeeded in the identification of hundreds of genes that largely belong to the metal-homeostasis network (Memon and Schroder 2009). To understand the genetics of metal accumulation and adaptation, the vast arsenal of resources developed in A. thaliana could be extended to one of its closest relatives that display the highest level of adaptation to high metal environments such as A. halleri and T. caerulescens. Further studies could also be carried out with fast growing, high biomass producing and economically important Brassica sps which can be used for both for phytoremediation and for biofuel and/or industrial oil production.

In this comminication I will describe recent advances in understanding the genetic and molecular basis of the metal induced gene expression in plants including the gene expression work which is being carried out in my laboratory on some metal accumulating plant species in Brassicaceae family.

While surveying the flora of Cu mining areas of Southeastern Anatolia, we discovered several endemic metal accumulator plants accumulated high amount of several metals as shown in Table 1.

Table1. Plant species surveyed from eastern Turkey for heavy metal accumulation.

Plant species	Metal
Brassica nigra	Cu
Trigonella velutina	Cd
Onosma sericeum	Cr
Rumex scutatus	Mn
Alyssum filifome	Ni
Centaurea consanguinea	Ni, Cr
Trifolium arvense	Cd
Medicago lupulina	Zn
Lamium amplexicaule	Ni
Calodophora sps	Fe, Zn, Cu, Ni

Interestingly a Brassica nigra ecoptype found from Diyarbakir site contained a very high amount of Cu in their shoots (around 700 ppm Cu in their leaves) (see Fig 1) (Memon, 2006). When plants from this ecotype were regenerated from callus culture and grown in soil culture containing 200 ppm Cu, the shoots accumulated x3 more Cu (700 ug/g D.W.) than roots (Yildizhan and Memon unpublished data).

This ecotype could be considered as a good candidate for Cu phytoremdiation (Memon, 2000). Our data showed that ~ 20000 µg Cu g-1 DW was accumulated in the shoots of B. nigra when grown at 500 µM Cu. The expression γ-ECs and PCS was also increased several times in shoots when plants were subjected to high Cu concentration (Memon et al. 2009). Specially the expression of key regulatory enzyme in glutathione pathway (γ-EC) was increased around 9 fold in the leaves when plants were subjected to 200µM Cu treatment (Fig 1).

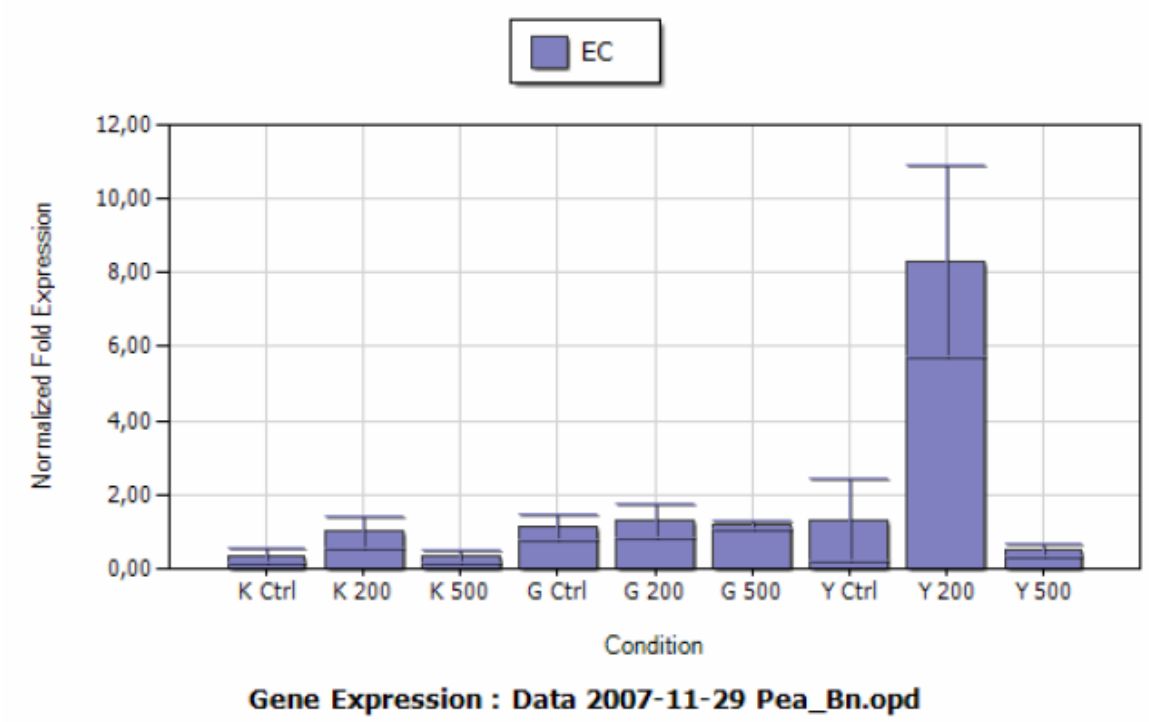

Fig.1. R-T(real time) PCR experiments showing γ-EC expression in roots (K), stems (G) and leaves (Y) of Brassica nigra grown in 0 (control), 200, and 500 µM Cu (Memon et al 2008).

Comparative transcriptome analysis was carried in order to find out the expression level of metal induced genes and transcriptome changes both in low and high Cu treated plants. Microarray analysis showed that some of the genes were up regulated (several hundred folds) and some were down regulated when plants were exposed to high Cu (Memon, 2009). The Cu accumulation capacity of Diyarbakir ecotype was determined and compared with other Brassica nigra ecotypes 6619, 6620 and 6630 obtained from different sites of Western Europe. In these comparative studies Brassica nigra Diyarbakir ecotype was found to be a super accumulator of Cu compare to other European ecotypes (Memon et al. 2009).

Microarray analysis showed several hundred folds up regulation of metal related genes including the genes involved in glutathione pathway, metal ATPase and ABC transporters in B. nigra when treated with 500 µM Cu. Currently we are carring out metabolomic studies with metal treated accumulator and non accumulator ecotypes of B. nigra by using HPLC-MS-MS inorder to identify metabolomic pattern in accumulator and non-accumulator ecotypes. Our aim is to identify specific metabolites which are upregulated and/or down regulated with Cu treatment in both ecotypes.

3.CONCLUSION

To understand fully the genetics of metal accumulation, the vast genetic resources developed in

A. thaliana must be extended to other metal accumulator species that display traits absent in this model species. A. thaliana microarray chips could be used to identify differentially expressed genes in metal accumulator plants in Brassicaceae. The

integration of resources obtained from model and wild species of the Brassicaceae family will be of utmost importance, bringing most of the diverse fields of plant biology together such as functional genomics, population genetics, phylogenetics, and ecology. Further development of phytoremediation technology requires an integrated multidisciplinary research effort that combines plant biology, genetic engineering, soil chemistry, soil microbiology, as well as agricultural and environmental engineering.

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